

ClinGen Rett and Angelman-like Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2

This version specified for the following genes: *CDKL5*, *FOXP1*, *MECP2*, *SLC9A6*, *TCF4*, *UBE3A*

Expert Panel Page: <https://clinicalgenome.org/affiliation/50022/>

Summary of changes in Version 2: Modifications to PP3 and BP4 (in silico prediction criteria) that affect splice site prediction, including thresholds to use for splice site prediction in silico tools.

Gene	Disease (MONDO ID)	Transcript
<i>CDKL5</i>	CDKL5 disorder (MONDO: 0100039)	NM_001323289.2
<i>FOXP1</i>	FOXP1 disorder (MONDO:0100040)	NM_005249.4
<i>MECP2</i>	Rett syndrome (MONDO:0010726)	NM_004992.3
<i>SLC9A6</i>	Christianson syndrome (MONDO:0010278)	NM_006359.2
<i>TCF4</i>	Pitt-Hopkins syndrome (MONDO:0012589)	NM_001083962.1
<i>UBE3A</i>	Angelman syndrome (MONDO:0007113)	NM_130838.2

Summary of ACMG-AMP Criteria for Rett /Angelman-like Syndromes

PATHOGENIC CRITERIA		
Criteria	Criteria Description	Specification
VERY STRONG CRITERIA		
PVS1	<p>Null variant in a gene where loss of function is a known mechanism of disease.</p> <ul style="list-style-type: none"> Use as defined by ClinGen SVI working group (PMID: 30192042) <i>FOXP1</i>: PVS1 is applicable up to p.S468. <i>MECP2</i>: PVS1 is applicable up to p.E472, for any frameshift variant that results in a read-through of the stop codon, for canonical splice site variants predicted to result in an out-of-frame product, and for canonical splice site variants or single in-frame deletions predicted to preserve the reading frame (exon 3). PVS1 is not applicable for initiation codons. <i>UBE3A</i>: PVS1 is applicable up to p.K841, for any frameshift variant that results in a read-through of the stop codon, for initiation codon variants, and for canonical splice site variants predicted to result in an out-of-frame product. <i>TCF4</i>: PVS1 is applicable up to p.E643, for any frameshift variant that results in a read-through of the stop codon, for canonical splice site variants predicted to result in an out-of-frame product, and for canonical splice site variants or single 	Disease-Specific

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	<p>in-frame deletions predicted to preserve the reading frame (exon 15).</p> <ul style="list-style-type: none"> • <i>SLC9A6</i>: PVS1 is applicable up to p.A563, for canonical splice site variants predicted to result in an out-of-frame product, and for canonical splice site variants or single in-frame deletions predicted to preserve the reading frame (exon 10). • <i>CDKL5</i>: Do not use PVS1 for truncating variants in <i>CDKL5</i> C-terminus (exons 19-21, or after p.P904) when using the historically used transcript (NM_003159.2). PVS1 is applicable up to p.R948 when using the major brain isoform which has an alternative C-terminus (NM_001323289.2), for canonical splice site variants predicted to result in an out-of-frame product, for canonical splice site variants or single in-frame deletions predicted to preserve the reading frame (exons 7, 10, 13), and for the non-coding <i>CDKL5</i> exon (exon 1) . 	
PS2_Very Strong	<p><i>De novo</i> (paternity confirmed) in a patient with the disease and no family history.</p> <ul style="list-style-type: none"> • ≥2 independent occurrences of PS2 • ≥2 independent occurrences of PM6 and one occurrence of PS2. 	Strength
PM6_VeryStrong	<p>Confirmed <i>de novo</i> without confirmation of paternity and maternity.</p> <ul style="list-style-type: none"> • ≥4 independent occurrences of PM6. Evidence from literature must be fully evaluated to support independent events. 	Strength
STRONG CRITERIA		
PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.	None
PS2	<i>De novo</i> (maternity and paternity confirmed) in a patient with the disease and no family history.	None
PS3	<p>Well-established <i>in vitro</i> or <i>in vivo</i> functional studies supportive of a damaging effect</p> <ul style="list-style-type: none"> • RNA studies that demonstrate abnormal splicing and an out-of-frame transcript • Do not use for canonical splice site variants and when PVS1 is used 	Disease-Specific
PS4	<p>The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.</p> <ul style="list-style-type: none"> • 5+ observations 	Strength

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PVS1_Strong	<p>Null variant in a gene where loss of function is a known mechanism of disease.</p> <ul style="list-style-type: none"> • <i>FOXP1</i>: PVS1_Strong is applicable for any truncating variant from p.S469 to p.Q480. • <i>UBE3A</i>: PVS1_Strong is applicable for any truncating variant from p.K842 to p.G850 and for canonical splice site variants that flank exons 7, 8 (in-frame exons). • <i>SLC9A6</i>: PVS1_Strong is applicable for any truncating variant from p.C564 to p.T601 and for canonical splice site variants that flank exon 3 (in-frame exon). 	Disease-Specific
PM4_Strong	<p>Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.</p> <ul style="list-style-type: none"> • PM4_Strong is applicable to stop-loss variants in <i>MECP2</i> and <i>UBE3A</i>. 	Disease-Specific
PM5_Strong	<p>Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.</p> <ul style="list-style-type: none"> • ≥2 different missense changes affecting the amino acid residue. • Do not apply PM1 in these situations. 	Strength
PM6_Strong	<p>Confirmed de novo without confirmation of paternity and maternity.</p> <ul style="list-style-type: none"> • ≥2 independent occurrences of PM6. • Evidence from literature must be fully evaluated to support independent events. 	Strength
PP1_Strong	<p>Co-segregation with disease in multiple affected family members</p> <ul style="list-style-type: none"> • ≥5 informative meioses • Note: individuals must have disease consistent with reported phenotype (even if on the mild end of spectrum of the disease) 	Strength
MODERATE CRITERIA		
PM1	<p>Located in a mutational hot spot and/or critical and well-established functional domain.</p> <ul style="list-style-type: none"> • <i>FOXP1</i>: (Forkhead: aa 181-275) • <i>TCF4</i>: (basic Helix-Loop-Helix domain (bHLH): aa 564-617) • <i>CDKL5</i>: (ATP binding region: aa 19-43; TEY phosphorylation site: aa 169-171) • <i>MECP2</i>: (Methyl-DNA binding (MDB): aa 90-162; Transcriptional repression domain (TRD): aa 302-306) • <i>UBE3A</i>: 3' cysteine binding site: aa 820 • Not to be used for <i>SLC9A6</i> 	Disease-Specific

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PM3	For recessive disorders, detected in trans with a pathogenic variant. <ul style="list-style-type: none"> Do not use 	NA
PM4	Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. <ul style="list-style-type: none"> <i>CDKL5</i>: Do not use for in-frame deletions/insertions in <i>CDKL5</i> C-terminus (exons 19-21, or after p.904) when using the NM_003159.2 transcript. <i>MECP2</i>: Do not use PM4 for in-frame deletions/insertions in the Proline-rich region of gene p.381-p.405) <i>FOXP1</i>: Do not use PM4 for in-frame deletions/insertions in the Histidine-rich region (p.37-p.57), Proline and Glutamine-rich region (p.58-p.86) and Proline-rich region (p.105-p.112). 	Disease-Specific
PM5	Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before. <ul style="list-style-type: none"> Applicable to all genes as written A Grantham or BLOSUM score comparison can be used to determine if the variant is predicted to be as or more damaging than the established pathogenic variant. 	None
PM6	Confirmed de novo without confirmation of paternity and maternity.	None
PVS1_Moderate	Null variant in a gene where loss of function is a known mechanism of disease. <ul style="list-style-type: none"> <i>FOXP1</i>: PVS1_Moderate is applicable for any truncating variant distal of p.Q480. <i>MECP2</i>: PVS1_Moderate is applicable for any truncating variant distal of p.E472. <i>UBE3A</i>: PVS1_Moderate is applicable for any truncating variant distal of p.G850. <i>TCF4</i>: PVS1_Moderate is applicable for any truncating variant distal of p.E643 and for single exon deletions that involve just non-coding exon 20. <i>SLC9A6</i>: PVS1_Moderate is applicable for any truncating variant between p.Y602 to p.A669 and any frameshift variant that results in a read-through of the stop codon. <i>CDKL5</i>: PVS1_Moderate is applicable for any truncating variant distal of p.R948 (when using the major brain isoform, NM_001323289.2) and for canonical splice site variants that flank exon 17 (in-frame exon). 	Disease-Specific

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PS4_Moderate	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. <ul style="list-style-type: none"> 3-4 observations 	Strength
PP1_Moderate	Co-segregation with disease in multiple affected family members <ul style="list-style-type: none"> 3-4 informative meioses Note: individuals must have disease consistent with reported phenotype (even if on the mild end of spectrum of the disease) 	Strength
SUPPORTING CRITERIA		
PP1	Co-segregation with disease in multiple affected family members <ul style="list-style-type: none"> 2 informative meioses Note: individuals must have disease consistent with reported phenotype (even if on the mild end of spectrum of the disease) 	Strength
PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease. <ul style="list-style-type: none"> Do not use 	N/A
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product <ul style="list-style-type: none"> For missense variants use REVEL with a score ≥ 0.75 For splice site variants use MaxEntScan, NNSPLICE and SpliceSiteFinder-like when all of the prediction programs support significant splicing alteration (significant splicing alterations defined as $\geq 15\%$ decrease to the natural splice site and $\geq 70\%$ gain in prediction strength of cryptic splice site) 	None
PP4	Phenotype specific for disease with single genetic etiology. <ul style="list-style-type: none"> See gene specific clinical phenotype guidelines 	Disease-Specific
PP5	Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation <ul style="list-style-type: none"> Do not use 	N/A
PVS1_Supporting	Null variant in a gene where loss of function is a known mechanism of disease. <ul style="list-style-type: none"> PVS1_Supporting is applicable for initiation codon variants in <i>CDKL5</i>, <i>FOXP1</i>, <i>SLC9A6</i> and <i>TCF4</i>. 	Disease-Specific
PS3_Supporting	Well-established in vitro or in vivo functional studies supportive of a damaging effect <ul style="list-style-type: none"> RNA studies that demonstrate abnormal splicing and an in-frame product (unless it affects an in-frame exon specified in the PVS1 section) See tables for <i>FOXP1</i>, <i>MECP2</i>, <i>CDKL5</i>, <i>TCF4</i>, <i>UBE3A</i> 	Disease-Specific

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	<ul style="list-style-type: none"> Not to be used for <i>SLC9A6</i> 	
PS4_Supporting	<p>The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.</p> <ul style="list-style-type: none"> Use for 2nd independent occurrence 	Strength
PM2_Supporting	<p>Absent/rare from controls in an ethnically-matched cohort population sample.</p> <ul style="list-style-type: none"> Use if absent, zero observations in control databases 	Strength
PM4_Supporting	<p>Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.</p> <ul style="list-style-type: none"> Smaller in-frame events (< 3 amino acid residues) unless they occur in a functionally important region (see PM1 for functionally important domains for each gene). 	Strength

BENIGN CRITERIA		
Criteria	Criteria Description	Specification
STAND ALONE CRITERIA		
BA1	<p>Allele frequency above 0.05%</p> <ul style="list-style-type: none"> Use large population databases (i.e. gnomAD) Use if variant is present at ≥ 0.0003 (0.03%) in any sub-population Use if allele frequency is met in any general continental population dataset of at least 2,000 observed alleles 	Disease-Specific
STRONG CRITERIA		
BS1	<p>Allele frequency greater than expected for disease (0.025%)</p> <ul style="list-style-type: none"> Use large population databases (i.e. gnomAD) Use if variant is present at ≥ 0.00008 (0.008%) and < 0.0003 (0.03%) in any sub-population Use if allele frequency is met in any general continental population dataset of at least 2,000 observed alleles 	Disease-Specific
BS2	<p>Observed in the heterozygous/hemizygous state in a healthy adult</p> <ul style="list-style-type: none"> 2 unaffected (related or unrelated) Het (<i>FOXG1</i>, <i>TCF4</i>), Hemi (<i>SLC9A6</i>), Het or Hemi (<i>CDKL5</i>, <i>MECP2</i>) 4 unaffected (related and maternally inherited or unrelated) Het (<i>UBE3A</i>) 	Strength

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BS3	Well-established in vitro or in vivo functional studies shows no damaging effect on protein function <ul style="list-style-type: none"> RNA functional studies that demonstrate no impact on splicing and transcript composition. It can be downgraded based on quality of data. Not applicable for these genes for other functional studies (see tables for other accepted functional studies) 	Disease-Specific
BS4	Lack of segregation in affected members of a family. <ul style="list-style-type: none"> Absent in a similarly affected family member, when seen in two or more families Need to confirm that the family member is 'affected with a neurodevelopmental phenotype consistent with the gene' at a minimum. 	Strength
BP5_Strong	Variant found in a case with an alternate molecular basis for disease <ul style="list-style-type: none"> ≥3 cases with alternate molecular basis for disease 	Strength
SUPPORTING CRITERIA		
BP1	Missense variant in gene where only LOF causes disease <ul style="list-style-type: none"> Do not use 	N/A
BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in cis with a pathogenic variant in any inheritance pattern. <ul style="list-style-type: none"> BP2 is applicable for <i>MECP2</i>, <i>TCF4</i>, <i>FOXP1</i> for in trans state BP2 is not applicable for <i>SLC9A6</i>, <i>UBE3A</i> and <i>CDKL5</i> for in trans state 	Disease-Specific
BP3	In-frame deletions/insertions in a repetitive region without a known function <ul style="list-style-type: none"> Inframe expansions or deletions in <i>FOXP1</i> repetitive regions: poly His (p.His47-p.His57), poly Gln (p.Gln70-p.Gln73) and poly Pro (p.Pro58-p.Pro61; p.Pro65-p.Pro69; p.Pro74-p.Pro80) 	Disease Specific
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product <ul style="list-style-type: none"> For missense variants use REVEL with a score ≤ 0.15 For splice site variants use MaxEntScan, NNSPLICE and SpliceSiteFinder-like when the majority of the prediction programs do not support significant splicing alteration (significant splicing alterations defined as ≥15% decrease to the natural splice site and ≥70% gain in prediction strength of a cryptic splice site) 	None
BP5	Variant found in a case with an alternate molecular basis for disease	Disease Specific

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	<ul style="list-style-type: none"> • <i>UBE3A</i>: variant should also be maternally inherited in the case with an alternate molecular basis for disease for this criteria to be used. • <i>SLC9A6</i>: the variant should be in the hemizygous state in the case with an alternate molecular basis for disease to be used. • Do not apply for any gene if variant is de novo 	
BP6	<p>Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation</p> <ul style="list-style-type: none"> • Do not use 	N/A
BP7	<p>A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.</p> <ul style="list-style-type: none"> • Defined “not highly conserved” regions in BP7 as those with PhastCons score <1 and/or PhyloP score <0.1 and/or the variant is the reference nucleotide in one primate and/or three mammal species. 	None
BS2_Supporting	<p>Observed in the heterozygous/hemizygous state in a healthy adult</p> <ul style="list-style-type: none"> • 1 unaffected (related or unrelated) Het (<i>FOXP1</i>, <i>TCF4</i>), Hemi (<i>SLC9A6</i>), Het or Hemi (<i>CDKL5</i>, <i>MECP2</i>) • 2 unaffected (related and maternally inherited or unrelated) Het (<i>UBE3A</i>) 	Strength
BS4_Supporting	<p>Lack of segregation in affected members of a family.</p> <ul style="list-style-type: none"> • Absent in a similarly affected family member • Need to confirm that the family member is ‘affected with a neurodevelopmental phenotype consistent with the gene’ at a minimum. 	Strength

Key: Disease-Specific: Disease-specific modifications based on what is known about disorders; **Strength:** Increasing or decreasing strength of criteria based on the amount of evidence; **N/A:** not applicable for genes; **None:** no changes made to existing criteria definitions.

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